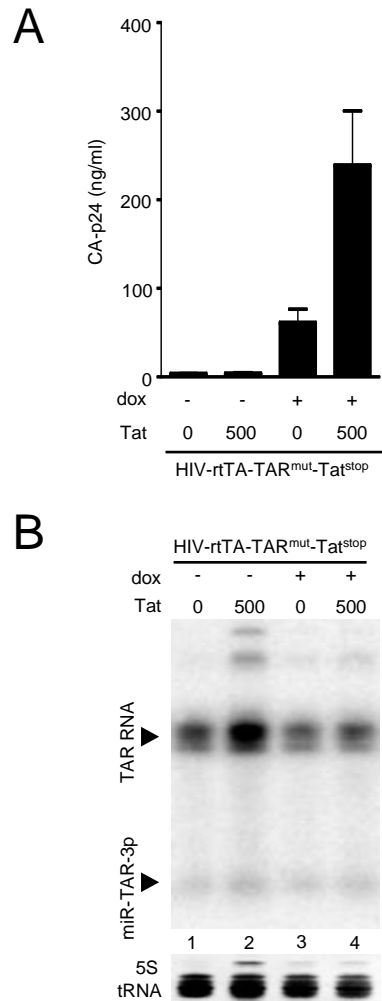
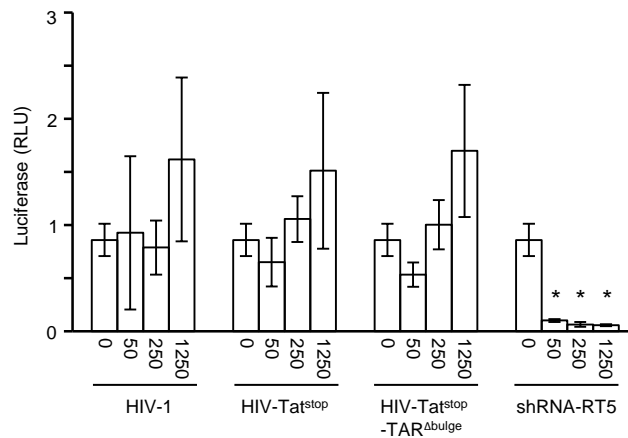


Supplementary Figure S1. Sp1 mutations inactivate the LTR promoter. 293T cells were transfected with luciferase-reporter constructs with the original (HIV-rtTA), TAR-deleted (Δ TAR) or Sp1-mutated (mutSp1) HIV-rtTA LTR promoter (35). Cells were co-transfected with an rtTA expressing plasmid and cultured with dox to activate transcription, as described previously (35). The intracellular luciferase level was measured after two days. Whereas TAR deletion does not affect promoter activity and luciferase production, the Sp1 mutations inactivate the LTR promoter and reduce luciferase production.



Supplementary Figure S2. Mutated TAR RNAs are processed independent of Tat. Tat-deficient HIV-rtTA constructs with a mutated TAR element (TAR^{mut}) were transfected into 293T cells. Cells were co-transfected with 500 ng pTat or the empty pcDNA3 plasmid (+ and - Tat, respectively) and cultured in the presence or absence of dox (+ and - dox, respectively). (A) The CA-p24 level was measured at 48 h. The mean \pm SD for 3 experiments is shown. (B) Small RNAs isolated from the transfected cells were analyzed by northern blotting using the miR-TAR-3p probe, as described for Fig. 3A. The higher TAR RNA and miR-TAR-3p signals in lane 2 are due to unequal RNA loading, as demonstrated by the higher ethidium bromide staining of the 5S rRNA and tRNAs in the lower panel.



Supplementary Figure S3. MiR-TAR-3p does not reduce expression of a reporter RNA transcript lacking the complementary target sequence. A luciferase reporter construct containing the target sequence for shRNA-RT5 in the 3' untranslated RNA region (42) was transfected into 293T cells together with 0 to 1250 ng HIV-1, HIV-Tat^{stop}, HIV-Tat^{stop}-TAR^{Δbulge} or a plasmid expressing shRNA-RT5. Luciferase production was measured 48 h after transfection. The mean ± SD for 4 experiments is shown. Statistical analysis using independent samples T-test analysis was used to compare the luciferase activity obtained without and with co-transfection of the HIV-1 or shRNA-RT5 constructs (*, P<0.01).